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Variants of Chromosome 9 With Additional Euchromatic Bands: Two Case Reports

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This paper documents a prenatal case of maternally inherited subtle duplication of chromosome 9 bands q21.1 to q21.2 and a second case with two G-bands within the 9qh region. Cytogenetic studies of these cases included G-banding, C-banding, fluorescent in situ hybridization (FISH) using chromosome 9 specific library probe, and the classical satellite probe. In both patients, the additional bands were G-bands and C-band negative. By FISH studies they were negative for the satellite heterochromatin probe and positive for the chromosome 9 painting probe. Therefore, the extra bands were presumably euchromatin and part of chromosome 9. The chromosome 9 variants and their possible mechanisms of origin are discussed. © 1996 Wiley-Liss, Inc.

KEY WORDS: chromosome 9, euchromatic normal variants, fluorescence in situ hybridization (FISH)

INTRODUCTION

Chromosome 9 is unique in the range of variants it exhibits. The variants include 9qh+, inv 9, an additional dark G-band within 9qh, on the short or long arm. These involve both heterochromatin and euchromatin. The additional euchromatic bands are interesting because they defy the generally held notion that euchromatic differences have clinical significance. Additional dark G-banded material on the long arm of chromosome 9 has been reported in normal carriers by Lin et al. [1994] and Jalal et al. [1990]. This paper documents the prenatal detection of a subtle maternally inherited duplication(9)(q21.1→q21.2). There are many reports of a single G-band within 9qh [Madan, 1978; Donlon and Magenis, 1981; Guanghui et al., 1984; Docherty and Hultén, 1985; 1993; Hoo, 1992; Roland et al., 1992; Verma et al., 1993]. Here we describe a case with two dark G-bands within the 9qh region.

CLINICAL REPORT

Patient 1

A 32-year-old pregnant woman underwent amniocentesis at 16 weeks of gestation because of an abnormal triple marker screen (0.56 MoM alpha-fetoprotein, 0.54 MoM estriol (uE3), and 2.21 MoM human chorionic gonadotropin). Chromosome analysis based on 15 cells from 15 colonies documented a subtle duplication of chromosome 9 in all the cells analyzed. Both parents' peripheral blood chromosomes were studied. The father's karyotype was normal. The fetus had inherited the dup(9) from the mother. The pregnancy was carried to term. A normal baby girl was delivered at 37 ⅐ weeks and had an Apgar score of 9/9. The baby weighed 3.75 kg, the length was 51.5 cm, and the head circumference was 34.5 cm.

Cytogenetic studies. The amniocytes and the mother's lymphocytes had an additional dark G-band on a chromosome 9 that appeared like a dup(9)(q21.1→q21.2) in GTG banding (Fig. 1a). The duplication was C-band negative (Fig. 1b). FISH using chromosome 9 specific DNA library probe (Oncor) and 9 classical satellite probe (Oncor) was performed as per the manufacturer's instructions. The chromosome 9 painting probe hybridized to the entire length of the chromosome 9 pair (Fig. 1c) and the satellite probe hybridized only to the constitutive heterochromatin region (9qh) (Fig. 1d). From these observations, it was concluded that the additional material was from chromosome 9, and it was not heterochromatin.

Patient 2

Chromosome analysis was performed on a 2-year-old girl. She was small for her age and had an atrial septal defect. A 9qh+ with two dark G-bands was observed in all the cells studied. All other chromosomes were normal. Family studies were not possible as the proposita had been adopted at birth.

Cytogenetic studies. GTG-, C-banding and FISH using a chromosome 9 specific library and 9 classical satellite DNA probe were performed. Two dark G-bands within the 9qh region were observed in all metaphases

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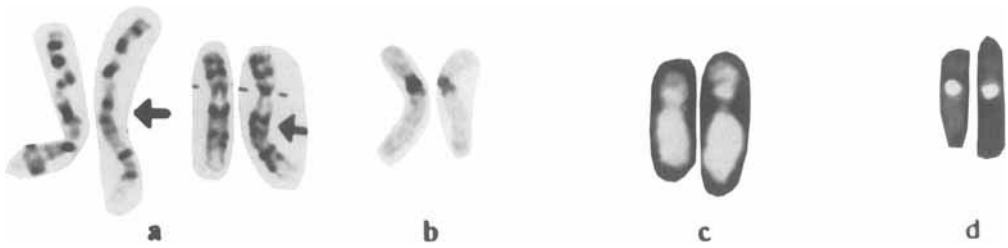

Fig. 1. Patient 1 dup(9)(q21.1q21.2). **a**: GTG banding shows a duplication of 9q21.1q21.2. **b**: In CBG banding, the additional material is C-band negative. **c**: FISH using chromosome 9 painting probe hybridized to the entire length of both chromosome 9's including the duplication. **d**: FISH using classical satellite heterochomatin probe hybridized to the qh region only and not the duplicated region.

analyzed (Fig. 2a). In C-banded preparations, these dark bands appeared as two gaps or two constrictions or stained uniformly (Fig. 2b). The FISH painting probe hybridized to the entire length of chromosome 9 pair. Therefore, the two G-bands were part of chromosome 9 and not from another chromosome (Fig. 2c); 9 classical satellite DNA probe hybridized to 9qh and no gaps were seen. However, two distinct constrictions were observed (Fig. 2d).

DISCUSSION

Chromosome 9 exhibits a wide spectrum of variants that range from the common heterochromatin variants to the rare euchromatic variant. The common heterochromatin variants, 9qh+ is found in about 8.2% of the population [Ferguson Smith, 1974] and a pericentric inversion of 9qh is present in about 1% of Finnish and British population [de la Chapelle et al., 1974; Madan and Bobrow, 1974]. These variants involve repetitive satellite DNA and hence are benign.

On the other hand, the rare variants involve euchromatin. Using chromosome banding methods, in situ hybridization and restriction enzyme digestion followed by banding, these have been shown to be part of chromosome 9 and not constitutive heterochromatin (9qh). They are the additional G-bands in the 9qh region, at 9p12 [Buckton et al., 1980; Archidiacono et al., 1984; Djalali et al., 1986; Spedicato et al., 1985; Sutherland and Eyre, 1981; Webb et al., 1989] and at 9q21. The euchromatic variants have been reported in apparently normal carriers and, therefore, lack phenotypic effects.

There are many reports of an extra G-band in the 9qh region [Madan, 1978; Donlon and Magenis, 1981; Guanghui et al., 1984; Docherty and Hulten, 1985, 1993; Hoo, 1992; Roland et al., 1992; Verma et al., 1993]. Several mechanisms for its origin have been postulated. They are "euchromatization" of the heterochromatin [Madan, 1978], amplification of unique sequences and direct or inverted duplication of the surrounding region [Macera et al., 1995; Luke et al., 1992; Docherty and Hultén, 1985]. The reason for the silencing of the genes in the euchromatin band is unknown, but could be due to position effect, as has been described in the Drosophila White gene. Another possibility is that the euchromatin band is inaccessible to transcription due to its chromatin structure. An example is the β-globin gene cluster in humans which is expressed exclusively in the erythroid cells. In cells where this gene is not expressed, the DNA is resistant to DNaseI digestion indicating that the DNA is tightly packed.

Patient 2 described in this paper has two G-bands within 9qh and is abnormal. However, the clinical significance of this cytogenetic finding is unknown since family studies were not possible. Extrapolating from reports on a single G-band, the two bands could similarly turn out to be a normal variant.

A variant resembling the one described in patient 1 was reported previously by Lin et al. [1994] and Jalal

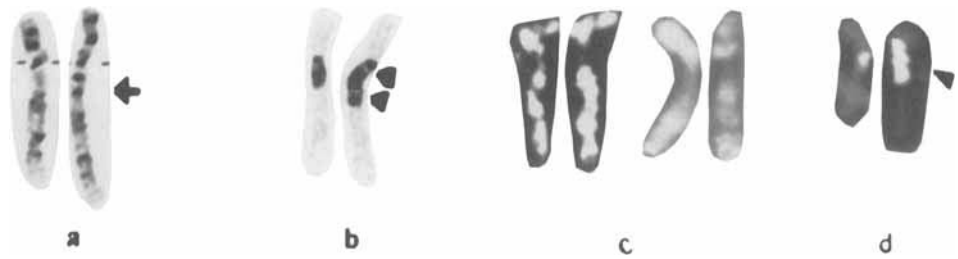

Fig. 2. Patient 2 9qh+ with two G-bands. **a:** GTG banding shows two dark bands in the qh region. **b:** In CBG banding the 9qh G-bands appear as gaps in staining. **c:** FISH using chromosome 9 painting probe hybridized to the entire length of both chromosome 9's. **d:** In FISH using classical satellite heterochromatin probe the two G-band in the 9qh region appear as constrictions.

et al. [1990]. A baby with hypoplastic lungs and fetal hydrops had a chromosome 9 with excess material involving 9q13q21 [Jalal et al., 1990]. The mother had the same variant 9. Lin et al. [1994] observed a subtle duplication of chromosome 9 [dup(9)(q21.1q21.2)] in two unrelated families. In one family, the index case had neuroblastoma while in the other the index case had developmental and mental retardation. The former had inherited this chromosome from his normal father and the latter from a normal mother. The duplicated material was a dark G-band that was C-band negative. In FISH studies, the chromosome 9 specific library probe hybridized to the additional band. However, the α- and β-satellite DNA probes specific for the centromere and 9qh did not hybridize to the band. Therefore, the duplication was part of chromosome 9 but was not constitutive heterochromatin. The growing evidence from the above reports is that these duplications are variants without apparent phenotypic effects.

Duplication of 9q21.2 to q22.3 is associated with multiple congenital anomalies and mental retardation syndrome [Kajii et al., 1987]. Therefore, it is important for de novo cases with additional material on proximal 9q to be characterized using both high resolution banding (HRB) and FISH-HRB to define the exact bands involved and FISH to rule out the possibility of an insertion from another chromosome.

Although bands 9p12, 9q21.1, 9q21.2, and 9q21.3 have been implicated in the unequal crossing over resulting in these variants [Macera et al., 1995], the definitive answer will come from molecular studies that will uncover the genetic makeup of these additional bands and their origin.

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